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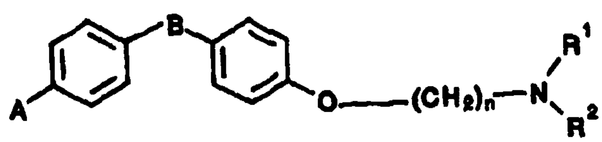
(54) Title: LTA_4 HYDROLASE INHIBITORS

(57) Abstract

The present invention provides compounds having structure (I) and pharmaceutically acceptable salts and stereoisomers thereof that are useful in the treatment of inflammatory diseases which are mediated by LTB_4 production, such as psoriasis, ulcerative colitis, IBD, and asthma.



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TITLE

LTA_4 HYDROLASE INHIBITORS

Field of the Invention

This invention relates generally to anti-inflammatory compounds and pharmaceutical compositions, and more particularly to anti-inflammatory compounds and compositions which are capable of inhibiting leukotriene A_4 hydrolase.

Background of the Invention

LTA_4 hydrolase is a requisite enzyme in the biosynthetic pathway leading to LTB_4 formation. LTB_4 is a proinflammatory compound. R. Lewis, et al., *N. Engl. J. Med.* 323, 645-655 (1990) have demonstrated that LTB_4 is a potent granulocyte agonist inducing chemotaxis, aggregation, degranulation, adherence and priming of inflammatory cells for induction by other agonists. Binding of LTB_4 to receptors is stereospecific with two distinct classes of binding sites. A. Lin, et al., *Prostaglandins* 28, 837-849 (1984). A high affinity site [$4\text{-}5 \times 10^{-10}$ M] mediates chemotaxis and chemokinesis while lower affinity sites [$0.6\text{-}5 \times 10^{-7}$ M] stimulate

granular secretion and oxidative burst. The LTB_4 receptor is associated with a GTP-binding protein that regulates affinity and transduces signals. T. Schepers, et al., *J. Biol. Chem.* 267, 159-165 (1992). Elevated LTB_4 levels have been reported for many diseases. Most prominently, elevated LTB_4 levels have been correlated to the pathology of inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis and in psoriasis. P. Sharon, et al., *Gastroent.* 86, 453-460; K. Lauritsen, et al., *Gastroent.* 95, 11-17 (1989); S. Brain, et al., *Br. J. Pharm.*, 83, 313-317 (1984). Other properties of LTB_4 which may contribute to disease processes are: stimulation of mucus secretion; stimulation of cytokine production; and the ability to act synergistically with other inflammatory mediators such as prostaglandins and cysteinyl leukotrienes thereby amplifying the inflammatory process.

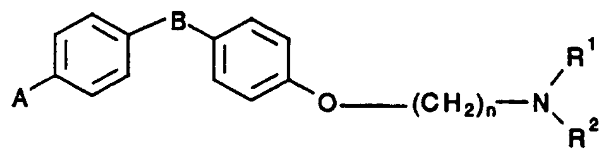
B. Samuelsson, et al., *J. Biol Chem.*, 264, 19469-19472 (1989) have shown that LTB_4 biosynthesis from arachidonic acid involves the action of 2 enzymes, 5-lipoxygenase [5-LO] and LTA_4 hydrolase. 5-LO transforms arachidonic acid to 5-HPETE and subsequent formation of LTA_4, which is an unstable allylic epoxide intermediate which is enzymatically hydrolyzed by LTA_4 hydrolase to form the dihydroxy acid LTB_4.

LTA_4 hydrolase is distinct from cytosolic and microsomal epoxide hydrolases based on strict substrate requirements, product formation [5(S),12(R) vs. 5(S),6(R)] for mouse liver cytosolic epoxide hydrolase, and lack of inhibition by inhibitors of cytosolic epoxide hydrolase. LTA_4 hydrolase appears to be ubiquitously distributed in mammalian tissues even in cell types that do not express 5-LO, suggesting the importance of transcellular metabolism of LTA_4. While peptidomimetic compounds such as bestatin and captopril

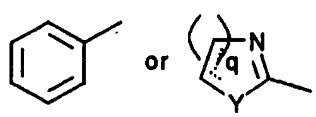
have been shown to exhibit LTA_4 hydrolase inhibitory activity, they are not able to satisfy the requirement of a small organic compound which is capable of cellular penetration. It would therefore be very advantageous to be able to provide low molecular weight inhibitors of LTB_4 biosynthesis which preferably exhibit oral activity in vivo at desirably low concentrations.

Summary of the Invention

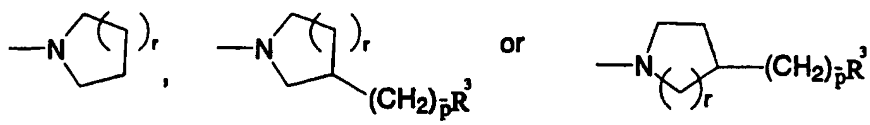
Applicants have now discovered that compounds having the structure:



and pharmaceutically acceptable salts and stereoisomers thereof possess LTA_4 hydrolase inhibitor activity wherein

A is 

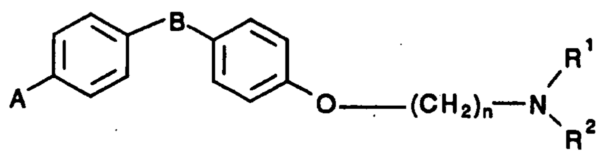
wherein ..... represents a single or double bond
q is 1 or 2, and
Y is -O-, -S-, $-CH_2-$, or -CH-
B is -O-, $-CH_2-$ or $-CH_2O-$
n is an integer from 2 to 4
R^1 is H or C_1 to C_4 alkyl
R^2 is $(CH_2)_m$ R^3 wherein m is an integer from 1 to 3
R^3 is CO_2R^4
R^4 is H alkyl, amino, alkylamino, dialkylamino
or NR^1R^2 is combined to form



wherein r is 1 or 2, p is 0 to 3 and R^3 is as defined above.

Detailed Description

In one of its embodiments, the present invention entails compounds having the structure:



and pharmaceutically acceptable salts and stereoisomers thereof, wherein A, B, R^1, R^2, and n are as defined above.

The compounds of the present invention, in several embodiments, may comprise a carboxylic acid or ester moiety. It will be appreciated by those of ordinary skill in the art that a compound of the present invention comprising an ester moiety is readily converted, *in vivo*, especially when administered orally, into its corresponding carboxylic acid form. The ester-containing compounds of the present invention are therefore prodrugs of their carboxylic acid form.

In another of its aspects, the invention entails pharmaceutical composition comprising a pharmacologically effective amount of at least one of

the compounds defined above and a pharmaceutically acceptable carrier.

In still another of its embodiments the present invention involves a method for treating a mammal exhibiting an LTB4 mediated inflammatory condition comprising administering to the mammal a pharmacologically effective amount of a compound of the invention.

The term "lower alkyl" means straight or branched chain alkyl having 1 to 6 carbon atoms such as methyl, ethyl, propyl, butyl, pentyl, hexyl and the branched chain isomers thereof. The term "lower alkoxy" means straight or branched chain alkoxy having 1 to 6 carbon atoms such as methoxy, ethoxy, propoxy, butoxy, pentoxy, hexoxy and the branched chain isomers thereof. The term "allyl" as used herein means the 1-propenyl radical, $-CH_2-CH_2=CH_2$. The term "halo" or "halogen" means fluoro, chloro, bromo, or iodo.

Included within the classes and subclasses of compounds embraced by this invention are isomeric forms of the described compounds including diastereoisomers, enantiomers and tautomeric forms of the described compounds. Pharmaceutically acceptable salts of such compounds are also included as well as pharmaceutically acceptable salts of such isomers and tautomers.

In the structures disclosed herein a bond drawn across a bond in a ring indicates that the bond can be to any available atom of the ring structure.

The expression "pharmaceutically acceptable salts" is intended to include those salts capable of being formed with the compounds of the present invention without materially altering the chemical structure or

pharmacological properties thereof. Such salts can be inorganic and organic cations or acid addition salts, including, but not limited to sodium, potassium, calcium, ammonium, alkylammonium, quaternary ammonium, triethanolamine, lysine, hydrochloride, hydrobromide, and others well known to those of ordinary skill in the art. The foregoing salts are prepared in the conventional manner by neutralization of the compounds of this invention with the desired base or acid.

The compounds of the present invention can be administered to a subject in such oral dosage forms as tablets, capsules, pills, powders, granules, elixirs or syrups, as well as aerosols for inhalation. Likewise, administration may be effected intravascularly, subcutaneously, or intramuscularly using dosage forms known to those of ordinary skill in the pharmaceutical arts. In general, the preferred form of administration is oral. An effective but non-toxic amount of the compound is employed in treatment. The dosage regimen utilizing the present compounds is selected in accordance with a variety of factors including the type, age, weight, sex and medical condition of the patient; the severity of the condition to be ameliorated; and the route of administration. A physician of ordinary skill can readily determine and prescribe a "pharmaceutically effective amount" of at least one of the compounds defined above, that is, the effective amount of the compound required to prevent, treat or arrest the progress of the condition. Dosages of the compounds of the present invention will range generally between 0.1 mg/kg/day to about 100 mg/kg/day and preferably between about 0.5 mg/kg/day to about 50 mg/kg/day when administered to subjects suffering from allergic or hypersensitivity reactions or inflammation. The compounds may also be administered transdermally or topically to treat proliferative skin conditions such

as psoriasis. The daily dosage may be administered in a single dose or in equal divided doses, for example, three to four times daily. The subject is typically a mammal and, in particular, a human patient.

As used herein the phrase "LTA_4 hydrolase inhibitor" means a compound that is capable of exhibiting an IC_{50} of less than 1 mM in an in vitro assay employing 10 μg/ml of LTA_4 hydrolase enzyme (specific activity 600 nMoles LTB_4/min/mg of enzyme) in the presence of 25 μM substrate (LTA_4) in a total reaction volume of 100 μl.

In the pharmaceutical compositions and methods of the present invention, at least one of the active compounds of the invention or a pharmaceutically acceptable salt thereof will typically be administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier materials") suitably selected with respect to the intended form of administration and consistent with conventional pharmaceutical practices. For example, the pharmaceutical compositions of this inventio ncan be administered as oral tablets, capsules, elixirs, syrups and the like For oral administration in the form of tablets or capsules, the active drug component may be combined with any oral non-toxic pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol and the like; for oral administration in liquid form, the active drug component may be combined with any oral non-toxic pharmaceutically acceptable inert carrier such as ethanol and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated in the mixture. Suitable binders include starch, gelatin, natural sugars, corn

sweeteners, natural and synthetic gums such as acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes. Lubricants for use in these dosage forms include boric acid, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methylcellulose, agar, bentonite, guar gum and the like.

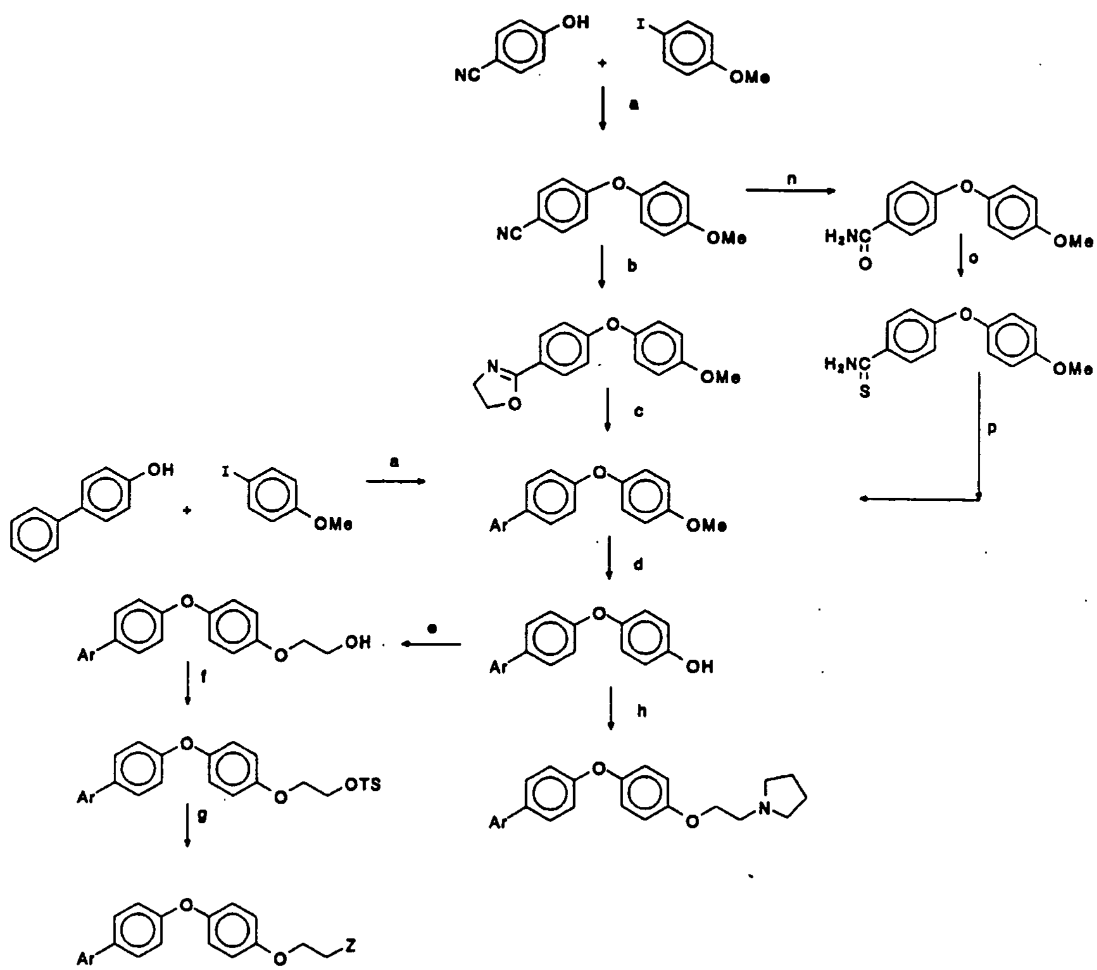
By virtue of their activity as LTA_4 hydrolase inhibitors, the compounds of the invention are useful in treating inflammatory conditions mediated by LTB_4 production in mammals such as psoriasis, contact and atrophic dermatitis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, multiple sclerosis, ankylosing spondylitis, arthritis, asthma and the like. Similarly, the compounds of the invention can be used in preventing recurring inflammatory attacks. A physician or veterinarian of ordinary skill can readily determine whether a subject exhibits the inflammatory condition. A preferred utility relates to treatment of ulcerative colitis.

The compounds of the invention are prepared from readily available starting materials by any of the following alternate processes in a conventional manner. The following reaction schemes describe methods which can be employed for preparing the compounds of the invention including starting materials, intermediates and reaction conditions. The following terms, as used herein, have the following definitions:

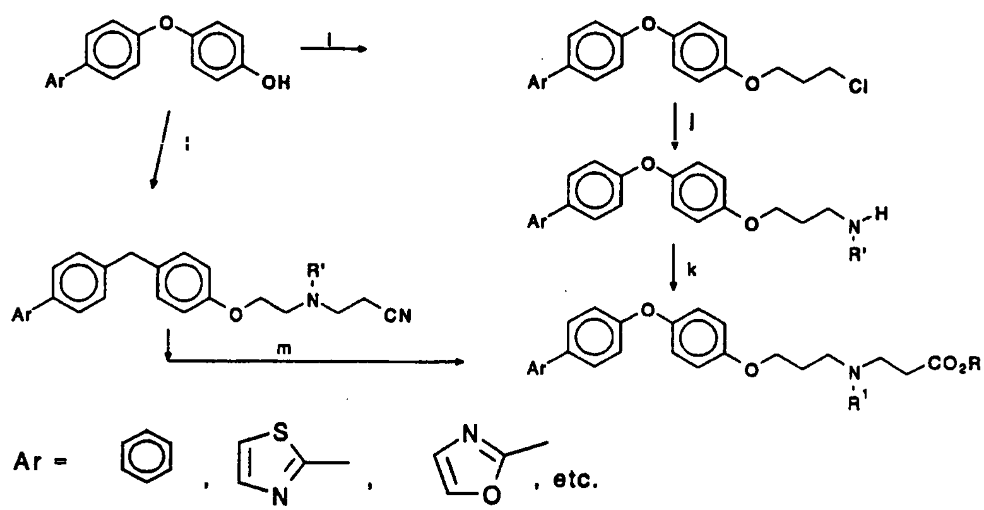
NMMO	N-methylmorpholine-N-oxide
Me	methyl
$SitBuMe_2$	t-butyldimethylsilyl
nBuLi	n-butyllithium
THF	tetrahydrofuran
Et_2O	diethyl ether

EtOH	ethyl alcohol
Pd/C	palladium on carbon
TFA	trifluoroacetic acid
Et_3SiH	triethylsilane
TBAF	tetrabutylammonium fluoride
DMF	dimethylformamide
nBu_4NBr	tetra-n-butylammonium bromide
TsCl	tosylchloride or p-toluenesulfonyl-chloride
TsO	tosylate or p-toluenesulfonate
MeOH	methyl alcohol
AcOH	acetic acid
Bn	benzyl
DEAD	diethylazodicarboxylate
Ph_3P	triphenylphosphine
MCPBA	metachloroperbenzoic acid
LAH	lithium aluminum hydride
TsOH	tosic acid or p-toluenesulfonic acid
LDA	lithium diisopropylamide
DSC	disuccinylcarbonate
nBuOH	n-butyl alcohol
TFAA	trifluoroacetic anhydride
Me_3SnN_3	trimethyl-tin azide
TMS	trimethyl silyl
Ac_2O	acetic anhydride
Ac	acetate
EtOAc	ethyl acetate
Hep	heptane

General Scheme



General Scheme
(continued)



<u>General Scheme</u>
(continued)

a) KOH, Cu°, 160°C-200°C

b) $ZnCl_2$, ethanolamine, 130°C

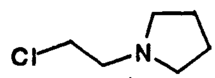
c) NiO_2, benzene, reflux

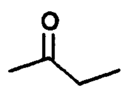
d) CH_2Cl_2, BBr_3, -78°C

e) Ethylene carbonate, DMF, nBu_4NBr, 140°C

f) TsCl, pyridine, CH_2Cl_2, 0°C

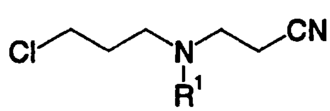
g) DMF, K_2CO_3, ZH, where Z is NR^1R^2 wherein R^1 and R^2 are as defined hereinbefore

h) DMF, K_2CO_3,  HCl, 80°C

i) , K_2CO_3, , 90°C

j) CH_3CN, H_2NR', 55°C

k) CH_2Cl_2, methylacrylate, room temp.

l)  , KOH, DMSO

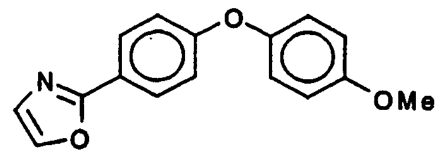
m) HCl

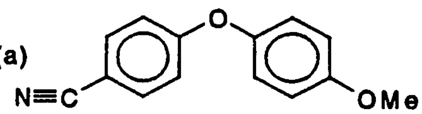
n) KOH, DMSO, tBuOH, reflux

o) Lawesson's reagent, toluene, reflux

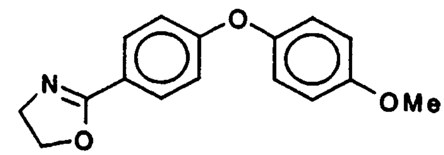
p) $(CO_2H)_2$, $ClCH_2CH(OMe)_2$, reflux

Example 1

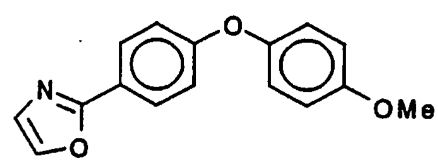
(c) 

(a) 

(a) A mixture of 4-iodobenzonitrile (5.06 g, 22 mmol), 4-methoxyphenol (2.72 g, 22 mmol), potassium carbonate (3.182 g, 22 mmol), and copper bronze (1.39, 22 mmol) in pyridine (120 ml) was heated to reflux under argon for 4 days. The reaction was allowed to cool to room temperature and concentrated in vacuo. The brown residue was acidified to pH = 1 with concentrated HCl and diluted with water. The mixture was extracted with EtOAc (2X) and the organic layers collected. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give a black/brown solid (4.56 g). The solid was purified by column chromatography (5% EtOAc/hexane followed by 10% EtOAc/hexane) to give a white solid (1.8 g). NMR spectrum is consistent with structure (a) above.

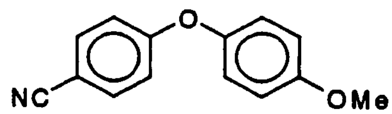
(b) 

(b) A mixture of fused $ZnCl_2$ (0.782 g, 5.3 mmol), the compound from step a (0.548 g, 2.2 mmol), and ethanolamine (15 ml) was heated to 130-140°C for 4 hours The reaction was diluted with CH_2Cl_2, and washed with water (2X) and brine. The organic layer was collected and dried over $MgSO_4$. Concentration in vacuo gave a white solid (0.71 g). The solid was purified by column chromatography (100 g silica gel, 5% $MeOH/CH_2Cl_2$ (500 ml)) gave the desired product as a white solid (0.29 g). NMR spectrum is consistent with structure (b) above.

(c) 

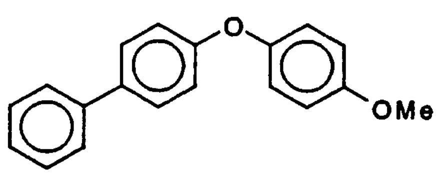
(c) A mixture of the compound of step b (0.149 g, 0.59 mmol) and NiO_2 (0.838 g, 8.9 mmol) in benzene (10 ml) was heated to reflux for 17 hours. The reaction was allowed to cool to room temperature and filtered through celite. Concentration of the filtrate gave a white solid (0.10 g). NMR spectrum is consistent with structure (c) above.

Example 2



A mixture of 4-cyanophenol (1.856 g, 15.4 mmol) and potassium hydroxide (0.868 g, 14.1 mmol) was heated to 140°C under argon. The resulting solution resolidified within 15 min. of heating. At this time, 4-iodoanisole (3.039 g, 12.8 mmol) was added followed by activated Cu (0.277 g) and the reaction mixture was heated to 170°C for 20 hours. The reaction was allowed to cool to room temperature and 10% NaOH added. The mixture was extracted with Et_2O (4 X 75 ml). The organic layers were collected, washed with brine and dried over $MgSO_4$. Concentration in vacuo gave a red/brown oil (0.68 g). The oil was purified by column chromatography (50 g silica gel; 5% EtOAc/hexane followed by 10% EtOAc/hexane) to give the product as a pale yellow solid (0.140 g). NMR spectrum is consistent with the structure above.

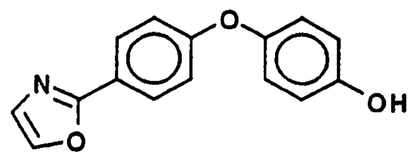
Example 3



The procedures described in Example 2 were repeated using 4-phenylphenol (4.366 g, 25.6 mmol) in place of 4-cyanophenol, and 4-iodoanisole (5.053, g, 21.4 mmol). The reaction was heated to 200°C for 3.5 hours. After

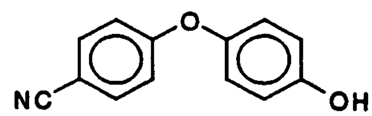
work-up, a pale yellow solid was collected. The solid was recrystallized from MeOH to give the desired product (1.03 g). NMR spectrum is consistent with the structure above.

Example 4



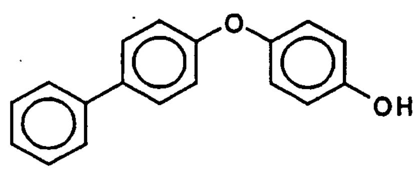
A solution of the compound of Example 1 (0.08 g, 0.3 mmol) in CH_2Cl_2 (2 Ml) was cooled to -78°C. A 1 M solution of BBr_3 in CH_2Cl_2 (0.66 ml) was added slowly under argon. The reaction was allowed to warm slowly to room temperature over 1.5 hours. The reaction was concentrated in vacuo and a mixture of water and CH_2Cl_2 was added to the residue. The organic layer was collected and washed with brine. Concentration in vacuo gave a brown oil (0.079 g). The oil solidified upon standing at room temperature. The solid was slurried with CH_2Cl_2 (3-5 ml) and the undissolved solid was collected by vacuum filtration to give a grey solid (0.047 g). NMR spectrum is consistent with the structure above.

Example 5



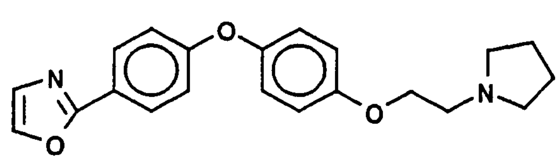
The procedures described in Example 4 were repeated using the compound of Example 2 (0.259 g, 1.2 mmol) in place of the compound of Example 1. After work-up, a blue solid was obtained as the desired product (0.262 g). NMR spectrum is consistent with the structure above.

Example 6



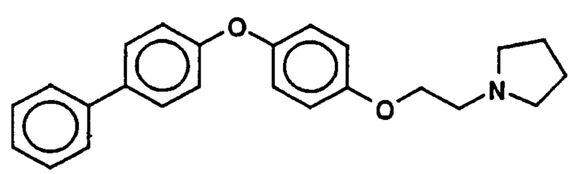
The procedures described in Example 4 were repeated using the compound of Example 3 (1.03 g, 3.7 mmol) in place of the compound of Example 1. Upon work-up, the desired product was obtained as a white solid (0.887 g). NMR spectrum is consistent with the structure above.

Example 7



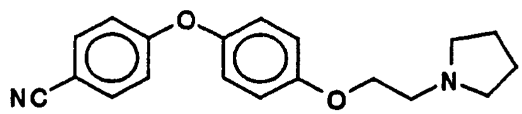
A mixture of the compound of Example 4 (0.04g, 0.16 mmol), potassium carbonate (0.120 g, 0.79 mmol), and 1-(2-chloroethyl)pyrrolidine hydrochloride (0.037 g, 0.19 mmol) in DMF (3 ml) was heated to 80°C (bath). After 21 hours of heating, the reaction was allowed to cool to room temperature and diluted with EtOAc (20 ml). The resulting solution was washed with water (2 X 20 ml) and brine (20 ml). The organic layer was collected, dried over $MgSO_4$, and concentrated in vacuo to give a white/yellow solid (0.04 g). The solid was purified by plate chromatography (5% $MeOH/CH_2Cl_2$) to give the desired product as a tan solid (0.022 g). Anal. calc'd for $C_{21}H_{22}N_2O_3$ + 0.2 H_2O: C, 71.25; H, 6.38; N, 7.91. Found: C, 71.03; H, 5.98; N, 7.80. M^+=350.

Example 8



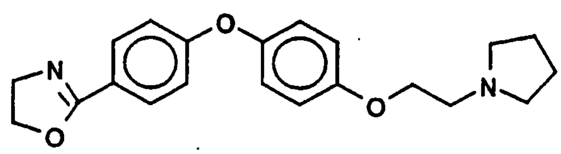
The procedures described in Example 7 were repeated using the compound of Example 6 (0.360 g, 1.4 mmol) in place of the compound of Example 4. After work-up, a yellow/white solid was obtained. The solid was further purified by slurrying with MeOH to give a cream-colored solid as the desired product (0.202 g). Anal. calc'd for $C_{24}H_{25}NO_2$ + 0.2 H_2O: C, 79.40; H, 7.05; N, 3.86. Found: C, 79.65; H, 7.11; N, 3.84. MH^+=360.

Example 9



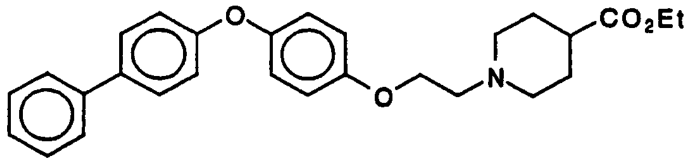
The procedures described in Example 7 were repeated using the compound of Example 5 (0.262 g, 1.2 mmol) in place of the compound of Example 4. After work-up, a yellow/brown oil was obtained as the desired product (0.260 g). NMR spectrum is consistent with the structure above.

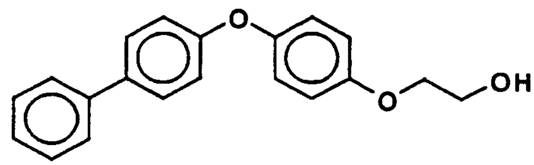
Example 10



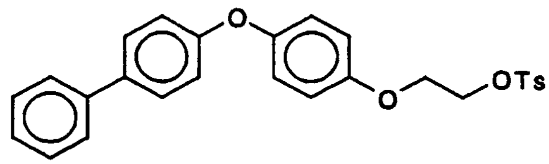
The procedures described in step b of Example 1 were repeated using the compound of Example 9 (0.087 g, 0.28 mmol) in place of the compound of Example 1 step a. After work-up, a yellow/white solid was obtained. The solid was further purified by column chromatography (95 $CHCl_3$: 5 EtOH: 0.5 NH_4OH) to give the desired product as a white solid (0.06 g). Anal. calc'd for $C_{21}H_{24}N_2O_3$ + 0.3 H_2O: C, 70.49; H, 6.93; N, 7.83. Found: C, 70.48; H, 7.02; N, 7.77. MH^+=353.

Example 11

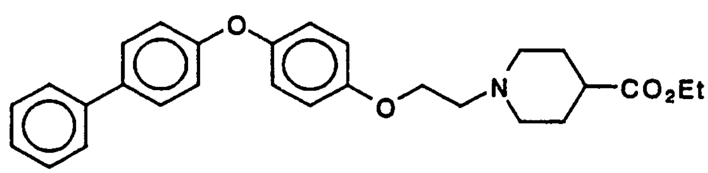


(a) 

(a) To a solution of the compound of Example 6 (0.411 g, 1.6 mmol) in DMF (5 ml) was added ethylene carbonate (0.255 g, 2.9 mmol) and nBu_4NBr (0.108 g, 0.31 mmol) under argon. The reaction was heated to 140-150°C (bath). After 8 hours, additional ethylene carbonate (0.041 g) was added to the reaction. The reaction was stirred at 140-150°C for an additional 16 hours before concentrating the reaction in vacuo. The resulting residue was dissolved in CH_2Cl_2 and washed with brine. The organic layer was collected, dried over $MgSO_4$, and concentrated in vacuo to give a light tan solid (0.570 g). The solid was recrystallized from EtOAc to give the desired product (0.210g). NMR spectrum is consistent with the structure (a) above.

(b) 

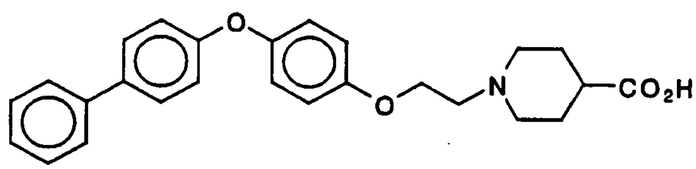
(b) A mixture of the compound from step a (0.085 g, 0.28 mmol) and TsCl (0.074 g, 0.36 mmol) in CH_3CN (1.5 ml) was cooled to 0°C. Triethylamine (0.15 ml, 1.1 mmol) was then added neat under argon. The reaction was allowed to stir at 0°C for 5 min. before removing the ice bath. The reaction was stirred at room temperature for 22 hours and then quenched with water. The resulting mixture was filtered and the desired product was collected as a tan solid. The solid was rinsed with CH_3CN/water (3:7) then allowed to air dry to give 0.107 g. The product was combined with 5 other previous runs and purified by column chromatography (3:1 hexane/EtOAc) to give the desired product as a white solid (0.142 g). NMR spectrum is consistent with the structure (b) above.

(c) 

(c) To a solution of the compound from step b (0.142 g, 0.3 mmol) in DMF (1.5 ml) was added ethyl isonipecotate (0.05 ml, 0.3 mmol) followed by potassium carbonate (0.220 g, 1.5 mmol). The reaction mixture was heated to 80°C (bath) under argon for 19.5 hours. The reaction was concentrated in vacuo and the residue was diluted with water (20 ml). The mixture was

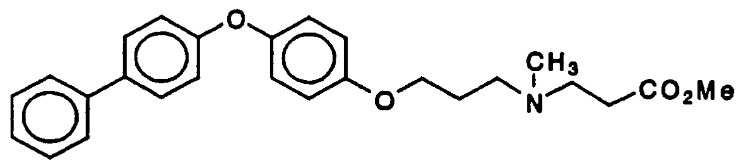
extracted with EtOAc (2 X 35 ml). The organic layers were combined, washed with brine, and dried over $MgSO_4$. Concentration in vacuo gave a white/yellow solid (0.134 g). The solid was purified by column chromatography (50 g silica gel, 1:1 EtOAc/hexane followed by 2:1 EtOAc/hexane) to give the desired product as a white solid (0.063 g). Anal. calc'd for $C_{28}H_{31}NO_4$: C, 75.48; H, 7.01; N, 3.14. Found: C, 75.28; H, 7.07; N, 3.09. M^+=445.

Example 12

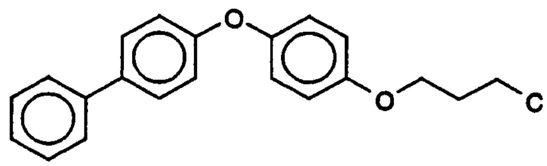


To a solution of the compound of Example 11 in distilled THF (5 ml) was added 6 M HCl. The reaction was heated to 85-95°C for 2 hours. The reaction was then concentrated in vacuo to give a white solid. The solid was purified by slurrying with ether. A white solid was collected by vacuum filtration (0.014 g). Anal. calc'd for $C_{24}H_{25}NO_2$ + 3.0 HCl + 1.0 H_2O: C, 57.31; H, 5.92; N, 2.57. Found: C, 57.37; H, 6.02; N, 2.25. M+=417.

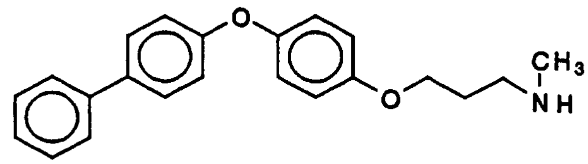
Example 13



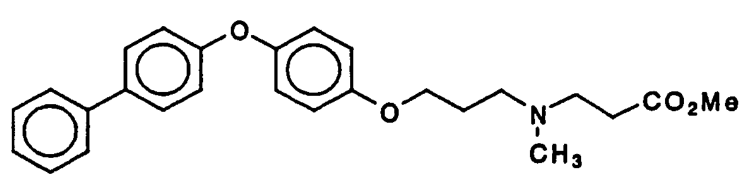
(a)



(a) To a solution of the compound of Example 6 (0.350 g, 1.3 mmol) in methyl ethyl ketone (4 ml) was added potassium carbonate (0.937 g, 6.7 mmol) followed by bromochloropropane (0.13 ml, 1.3 mmol) under argon. The resulting mixture was heated to 85-90°C (bath) for 21.5 hours, then heated to 95°C for 1.5 hours. The reaction was poured into a separatory funnel containing water (25 ml) and extracted with EtOAc (2 X 40 ml). The organic layers were combined, washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a yellow/white solid (0.442 g). The solid was purified by column chromatography (75 g silica gel, 5:1 hexane/EtOAC (500 ml)) to give the desired product as a white solid (0.324g). NMR spectrum is consistent with structure (a) above.

(b) 

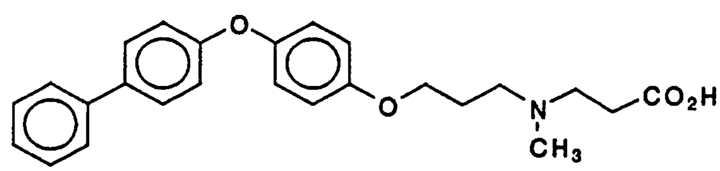
(b) To a mixture of the compound from step a (0.324 g, 0.96 mmol) in CH_3CN (6 ml) was added H_2NMe (8 ml, 95.6 mmol). Upon addition of H_2NMe, a white solid precipitated out of the mixture. The mixture was heated to 55°C (bath) for 8.5 hours. At this time, additional H_2NMe (2 ml) was added to the reaction. The reaction was stirred for another 16 hours at room temperature then heated to 55°C for 3 hours. The reaction was concentrated in vacuo and extracted with EtOAc (2 X 20 ml). The organic layer was collected, cooled to 0°C, and acidified to pH 1 with 6 M HCl. At this point, no solid was observed to precipitate out of solution. The solution was therefore basified to pH 12 with 10% NaOH and extracted with EtOAc (2 X 50 ml). The organic layer was collected and dried over $MgSO_4$. Concentration in vacuo gave a white solid. The solid was slurried with EtOAc and collected by vacuum filtration as the desired product (0.224 g). NMR spectrum is consistent with the structure (b) above.

(c) 

(c) To a solution of the compound of step b (0.224 g, 0.70 mmol) in CH_2Cl_2 (2 ml) was added methyl acrylate (0.08 ml, 0.91 mmol). The reaction was stirred at room temperature over 48 hours. At this time, additional

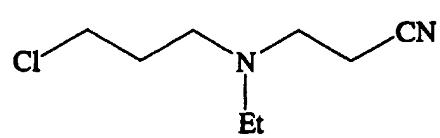
methyl acrylate was added (0.04 ml) to the reaction. The reaction was stirred for another 3 hours, then concentrated under a stream of N_2 to give a white/yellow solid. The solid was purified by column chromatography (50 g silica gel, 10% $MeOH/CH_2Cl_2$ to give the desired product as a white solid (0.200 g). Anal. calc'd for $C_{26}H_{29}NO_4$: C, 74.44; H, 6.97; N, 3.34. Found: C, 74.11; H, 6.85; N, 3.21. M^+=419.

Example 14



The compound of Example 13 (0.1 g) was treated with 6 M HCl under the same reaction conditions as those described in Example 12 to give the desired product as a white solid (0.073 g). $C_{25}H_{28}NO_4$ + 1.0 HCl + 0.8 H_2O: C, 65.80; H, 6.54; N, 3.07. Found: C, 65.71; H, 6.25; N, 2.81. MH^+=406.

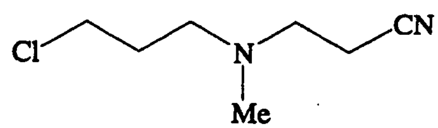
Example 15



200 mL of 2N ethylamine in methanol (0.4 mol), 10 mL (0.15 mol) acrylonitrile and 35 mL (0.25 mol) triethylamine were stirred in 100 mL methanol at 25°C for 21 hours. The mixture was concentrated and used without further purification. This was stirred in 70

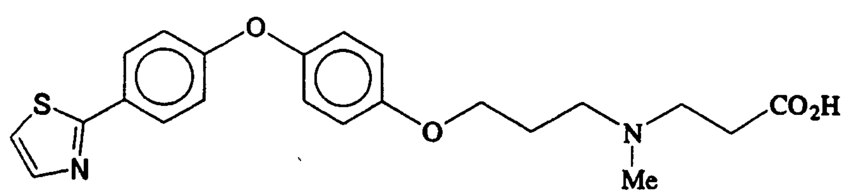
mL DMF with 44 mL (0.44 mol) 1-bromo-3-chloropropane and 25 mL (0.18 mol) triethylamine at 40°C for 5 hours and at 25°C for 15 hours. The mixture was poured into water and ether and the ether layer was washed with 2N HCl. The acid layer was washed with ether, made basic (>pH 10) with 45% KOH and extracted twice with ether. The ether extracts were dried over Na_2SO_4 and concentrated to provide the desired compound (21.7 g, 0.124 mol) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.04 (t,3H), 1.88 (m, 2H), 2.40-2.65 (m, 6H), 3.65 (t, 2H).

Example 16

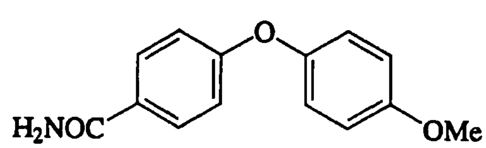


The compound was prepared as described for Example 15, using a solution of methylamine in place of ethylamine.

Example 17

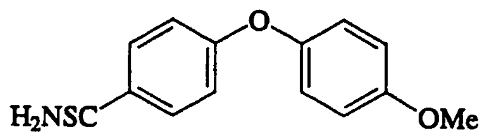


(a)

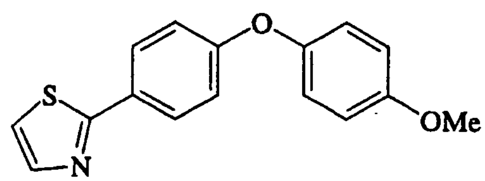


(a) To a suspension of the product from Example 2 (10.0 g, 44 mmol) in t-butanol (80 mL) was added 30 mL dimethylsulfoxide (DMSO) and powdered KOH (9.1 g, 162

mmol). The mixture was heated at reflux for 2 hours. The mixture was cooled and diluted with water (100 mL). The white solid precipitate was collected by filtration and washed with water (4 X 150 mL). The solid was dried in vacuo to give 9.6 g (89%) of (a): mp 195-196°C.

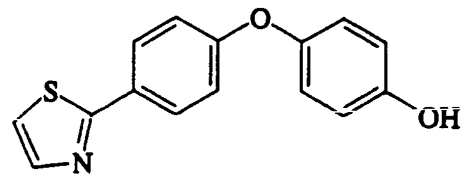
(b) 

(b) To a suspension of the compound from step (a) (4.2 g, 17.3 mmol) in toluene (80 mL) was added Lawesson's reagent (7.0 g, 17.3 mmol). The mixture was heated at reflux for 3 hours, cooled and concentrated in vacuo. The residue was chromatographed on silica gel (1:1 hexane/ethyl acetate) to give 2.2 g (49%) of (b).

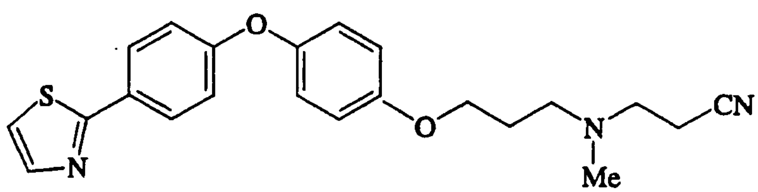
(c) 

(c) A mixture of oxalic acid (590 mg, 6.5 mmol) and chloroacetaldehyde dimethyl acetal (0.75 mL, 6.5 mmol) was heated at reflux for 1 hour. The oil bath was removed for 10 minutes and the compound from step (b) (1.7 g, 6.5 mmol) was added. The resulting mixture was heated at reflux for 2 hours. The mixture was cooled to room temperature and 30% HCl (3.5 mL) was added. The mixture was heated at reflux for 10 minutes, cooled and diluted with water. The reaction mixture was extracted with CH_2Cl_2 (3 X 10 mL). The organic solution was dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed (3:1 hexane/ethyl acetate)

to give the 925 mg (50%) of (c) as a crystalline solid: mp 92-93 °C; Anal. calcd for $C_{16}H_{13}NO_2S$: C, 67.82; H, 4.62; N, 4.94. Found: C, 67.66; H, 4.50; N, 4.86.

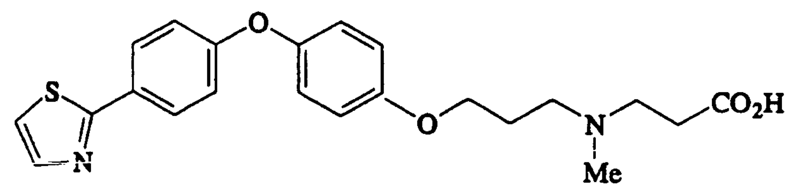
(d) 

(d) To a solution of the compound from step (c) (280 mg, 1.0 mmol) in CH_2Cl_2 (3 mL) at -78°C was added boron tribromide (1.8 mL of a 1M solution in CH_2Cl_2). The solution was kept at -78°C for 1 h and then warmed to room temperature over 2 hours. The reaction solution was diluted with water and extracted with CH_2Cl_2 (2 X 20 mL). The combined organic solution was dried (Na_2SO_4) and concentrated in vacuo to give 210 mg (78%) of (d).

(e) 

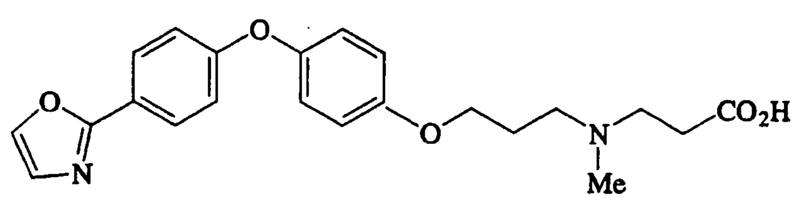
(e) To a suspension of powdered KOH (63 mg, 1.1 mmol) in DMSO (1 mL) as added via canula a solution of the compound from step (d) (200 mg, 0.74 mmol) in DMSO (2 mL). The mixture was stirred at room temperature for 5 min and the product of Example 16 (118 mg, 0.74 mmol) in DMSO (1 mL) was added via canula. The reaction mixture was heated at 45°C for 4 hours. The mixture was cooled to room temperature and partitioned between water and ether (15 mL). The aqueous solution was extracted with ether (2 X 10 mL). The combined organic solution was dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed (ethyl acetate) to give

112 mg (39%) of (e) as a crystalline solid: mp 63-64 °C.

(f) 

(f) A solution of the compound from step (e) (100 mg, 0.25 mmol) in 6N HCl (2 mL) was heated at 90°C for 17 hours. The solution was cooled to room temperature and brought to pH 8 with 10% NaOH. The aqueous solution was extracted with CH_2Cl_2 (3 X 15 mL). The organic solution was concentrated in vacuo. The residue as chromatographed on silica (85:14:1 CH_2Cl_2/MeOH/NH_4OH) to give 40 mg (39%) of (f) as a crystalline solid: mp 143-144°C.

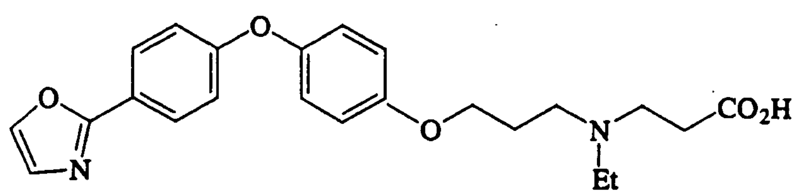
Example 18



The product from Example 4 (434 mg, 1.7 mmol), the product from Example 16 (305 mg, 1.9 mmol) and powdered KOH (158 mg, 2.8 mmol) were stirred in 20 mL DMF at 50°C for 12 hours. The mixture was cooled and diluted with 75 mL H_20. The aqueous base was separated and extracted with 3 X 25 mL methyl t-butyl ether (MTBE). The combined organic phases were dried ($MgS0_4$) and concentrated to afford the crude product as a brown oil. The crude nitrile was dissolved in 5 mL MTBE and 5 mL concentrated HCl was added. The MTBE was

distilled from the reaction and an additional 2 mL concentrated HCl was added. The reaction was heated to 95°C for 24 hours. After cooling, the mixture was diluted with 50 mL H_20 and neutralized with a saturated $NaHC0_3$ solution. The aqueous phase was extracted with 4 X 15 mL CH_2Cl_2 and the extracts dried ($MgS0_4$) and concentrated to afford a yellow oil. The oil was dissolved in 1 mL methanol and 3M ethanolic HCl was added until a precipitate formed. The tan solid was filtered and dried: Anal. calcd for $C_{22}H_{24}N_2O_5$•1.5 HCL•1.0 H_2O: C. 56.32; H, 5.91; N, 5.97; Cl, 11.34. Found: C, 56.61; H, 5.75; N, 5.32; Cl, 11.55.

Example 19



The above compound was prepared in the same manner as Example 18 substituting the product from Example 15 in place of the product of Example 16. The HCl salt was isolated as a tan solid: Anal. calcd for $C_{23}H_{26}N_2O_5$•1.25 HCl•1.0 H_2O: C, 58.27; H, 6.22; N, 5.91; Cl, 9.35. Found: C, 58.16; H, 6.25; N, 5.26; Cl, 9.14.

LTA Hydrolase Methods

The following Table presents data demonstrating the pharmacological activity of the LTA hydrolase inhibitors of the present invention. One or more of three different assays, (1) an in vitro LTA hydrolase enzyme assay, (2) a human whole blood assay utilizing calcium ionophore stimulation, and (3) a murine ex vivo assay utilizing calcium ionophore stimulation were

employed to determine the level of LTA hydrolase inhibitor activity.

Recombinant Human LTA Hydrolase Assay for LTA Hydrolase Inhibitor Activity

Compounds of the present invention were tested for LTA hydrolase inhibitor activity against recombinant human LTA hydrolase (rhLTAH). Recombinant human LTA hydrolase-encoding vectors were prepared and used to express rhLTAH essentially as described by J. Gierse, et al., *Protein Expression and Purification*, 4, 358-366 (1993). Briefly, LTA hydrolase encoding DNA was amplified by polymerase chain reaction using a pair of oligonucleotide primers based on the nucleotide sequence from the 5'-end, and the complement of the 3'-end, of the coding region of the LTA hydrolase gene, the nucleotide sequence of which gene is known. (See, C. Funk, et al., Proc. Natl. Acad. Sci. USA 84, 6677-6681 (1987)). A λgt11 human placental cDNA library (Clonetech, Palo Alto, CA) provided the nucleic acid template. The LTA hydrolase encoding region had a length of about 1.9 kb. The amplified 1.9 kb DNA was isolated and cloned into the genomic baculovirus, *Autographa californica* nuclear polyderosis virus (AcNPC) DNA, and the baculovirus expression vector was transfected into Spodoptera frugiperda Sf-9 cells employing the calcium phosphate co-precipitation method (see, M. Summers, et al., Tex. Agric. Exp. Stn. Bull. 1555, 1-57 (1987). Recombinant LTA_4 hydrolase enzyme was purified from the transfected Sf-9 cells essentially as described by J. Gierse, et al., supra.

One or more predetermined amounts of a compound of the invention were incubated in assay buffer (0.1 M potassium phosphate, 5 mg/ml fatty acid free BSA, 10% DMSO, Ph 7.4) for 10 minutes at room temperature with

250 ng of recombinant $hLTA_4H$ to allow binding, if any, between the enzyme and inhibitor. The stock enzyme solution was 1 mg/m. LTA_4 hydrolase, 50 Mm Tris, Ph 8.0, 150 Mm NaCl, 2.5 Mm beta-mercaptoethanol, 50% glycerol. The specific activity of the enzyme was about 650 Nmoles/min/mg. LTA_4 (i.e., substrate) was prepared from the methyl ester of LTA_4 (Biomol, Inc., Plymouth Meeting, PA) by treating the methyl ester with 30 molar equivalents of LiOH at room temperature for 18 hours. The LTA_4 substrate in its free acid form was kept frozen at -80°C until needed. LTA_4 (free acid) was thawed and diluted in assay buffer (minus DMSO) to a concentration of 350 ng/ml and 25 μl (8ng) of LTA_4 substrate was added to the reaction mixture (total volume of reaction mixture = 200 μl at time zero. Each reaction was carried out at room temperature for 10 minutes. The reaction was stopped by diluting 25 μl of the reaction mixture with 500 μl of the assay buffer without DMSO. LTA_4 was quantified in the diluted sample by a commercially available enzyme-linked immunoassay [Caymen Chemical Col. Ann Arbor, MI] using the method recommended in the manufacturer's instructions and compared to the amount of LTA_4 produced in a negative control (i.e., essentially identical conditions except without addition of an inhibitor compound). The IC_{50} was routinely calculated from the data produced.

LTB_4 and Thromboxane Production by Calcium Ionophore Stimulated Human Blood for LTB_4 Hydrolase Inhibitor Activity

Human blood, collected in heparin-containing Vacutainer tubes, was diluted 1:4 with RPMI-1640 media and 200 μl of the diluted blood was added into each of a 96-well microtiter plate. One or more concentrations of the leukotriene A_4 hydrolase inhibitor compounds being tested were prepared (diluted in DMSO) and 2 μl added

and gently mixed with the diluted whole blood. After incubating for 15 minutes at 37°C in a humidified incubator, calcium ionophore A13187 (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 20 mcg/ml and the incubation continued under the same conditions for an additional 10 minutes to allow LTB_4 formation. The reaction was terminated by centrifugation (833 g, 10 minutes at 4°C) and supernatant were analyzed for LTB_4 and thromboxane by commercially available enzyme-linked immunoassays (Caymen Chemical Co., Ann Arbor, MI) according to the manufacturer's instructions. The IC_{50} of each test compound was determined from the amount of inhibition of LTB_4 production as compared to an essentially identical assay in which no inhibitor compound was present.

Ex Vivo LTB_4 and Thromboxane Production by Calcium Ionophore Stimulated Mouse Blood for LTB_4 Hydrolase Inhibitor Activity

Leukotriene A_4 hydrolase inhibitor compounds of the present invention were diluted to a predetermined concentration in phosphate buffered saline containing 2% DMSO and 1% Tween 80. The compounds were administered by oral gavage to adult male outbred mice weighing approximately 20-30 gm at a dose of 10 mg/kg body weight. (Compounds given at a dose of 50 mg/kg body weight are designated in following Table by the symbol, *) Sixty (60) minutes after administration of an LTA_4 inhibitor compound of the invention, blood was collected (into heparin-containing tubes) from the retroorbital sinus. The heparinized blood was added to the wells of a microtiter plate along with an equal volume of RPMI-1640 media, and calcium ionophore A23187 was added to a final concentration of 20 mcg/ml. The mixture was incubated for 10 minutes at 37°C in a

humidified incubator. The reaction was terminated by centrifugation (833 g. 10 minutes at 4°C). Supernatant were analyzed for LTB_4 and thromboxane by commercially available enzyme-linked immunoassays [Caymen Chemical Co., Ann Arbor, MI] in accordance with the manufacturer's instructions. The percent inhibition was determined by comparison to animals treated identically except that the solution administered by oral gavage was devoid of inhibitor compound.

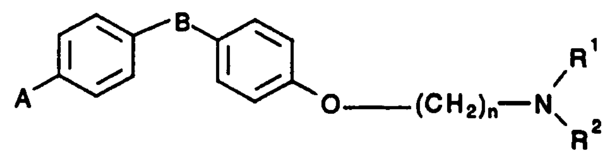
LTA_4 HYDROLASE INHIBITOR ACTIVITY

Ex.#	Recombinant Human LTA_4 Hydrolase Assay IC_{50} (μM)	Inhibition of Calcium Ionophore-induced LTB_4 Production in Human Blood IC_{50} (μM)	Murine Ex Vivo LTB_4 Inhibition %I LTB_4/at 1 hour after administration of 10mg/kg
7	0.43	0.55	93%
8	0.0066	0.14	57%
10	0.59	0.55	83%
11	0.34	0.72	90%
12	-	0.22	87%
13	0.55	0.79	63%
14	< 0.0005	0.19	78%
17	0.95	0.072	87%
18	0.027	0.19	94%
19	0.34	0.24	93%

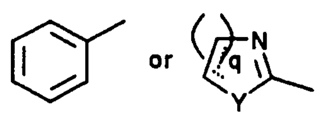
"-" means Not Determined

What is claimed is:

1. A compound having the structure:



wherein:

A is 

wherein ..... represents a single or double bond;

q is 1 or 2, and

Y is -O-, -S-, -CH- or $-CH_2-$

B is -O-, $-CH_2-$ or $-CH_2O-$

n is 2 to 4

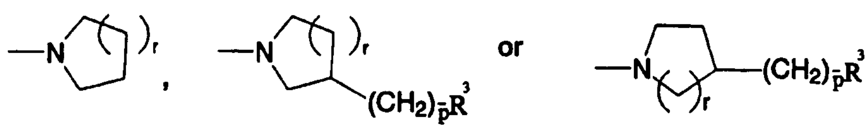
R^1 is H or C_1 to C_4 alkyl

R^2 is $(CH_2)_m$ R^3 wherein m is 1 to 3

R^3 is CO_2R^4

R^4 is H, alkyl, amino, alkylamino, dialkylamino;

or NR^1R^2 is combined to form

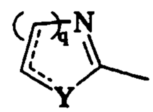


wherein r is 1 or 2, p is 0 to 3 and R^3 is as defined above.

2. The compound of claim 1 wherein B is O.

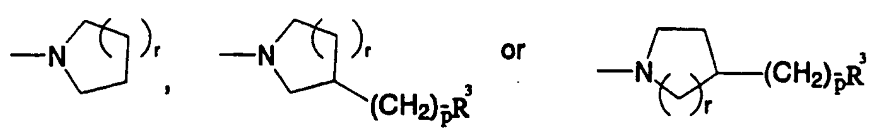
3. The compound of claim 2 wherein A is phenyl.

4. The compound of claim 2 wherein A is

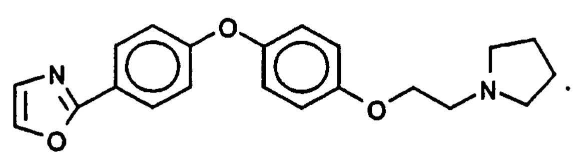


5. The compound of claim 2 wherein Y is -O- and q is 1.

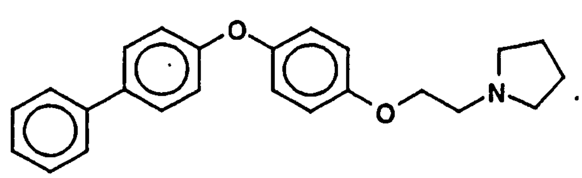
6. The compound of claim 2 wherein NR^1R^2 is combined to form



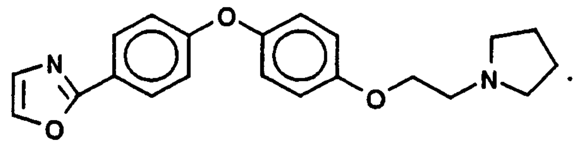
7. The compound of claim 1 having the structure:



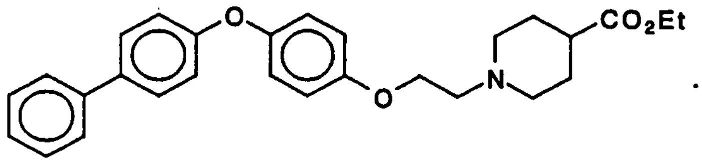
8. The compound of claim 1 having the structure:



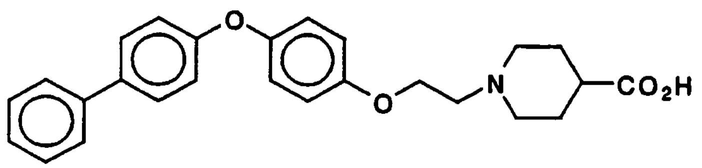
9. The compound of claim 1 having the structure:



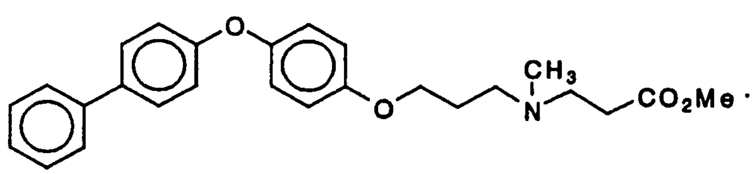
10. The compound of claim 1 having the structure:



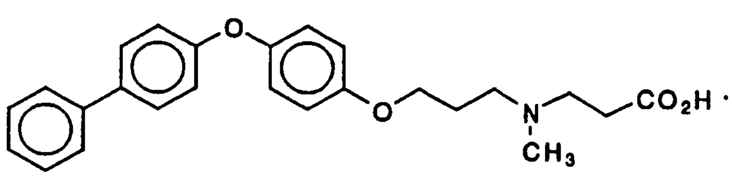
11. The compound of claim 1 having the structure:



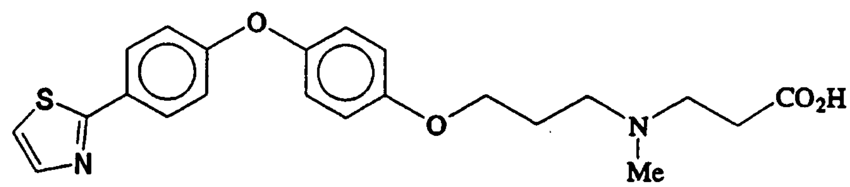
12. The compound of claim 1 having the structure:



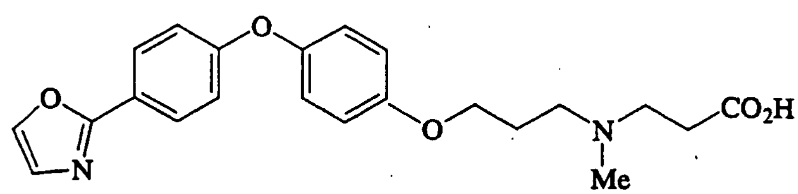
13. The compound of claim 1 having the structure:



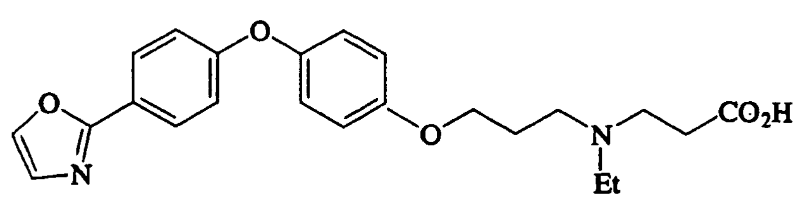
14. The compound of claim 1 having the structure:



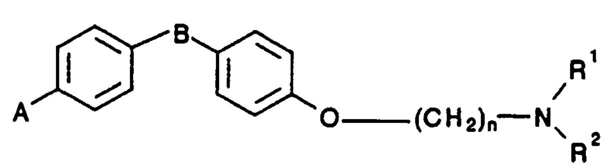
15. The compound of claim 1 having the structure:



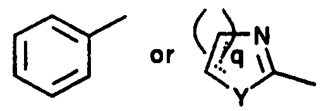
16. The compound of claim 1 having the structure:



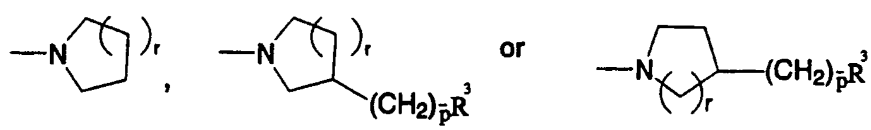
17. A pharmaceutical composition comprising compound having the structure:



or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable carrier, wherein

A is 

wherein ..... represents a single or double bond
q is 1 or 2, and
Y is -O-, -S- or -CH-
B is -O-, $-CH_2-$ or $-CH_2O-$
n is 2 to 4
R^1 is H or C_1 to C_4 alkyl
R^2 is $(CH_2)_m$ R^3 wherein n is 1 to 3
R^3 is CO_2R^4
R^4 is H alkyl, amino, alkylamino, dialkylamino or NR^1R^2 is combined to form

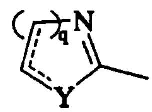


wherein r is 1 or 2, p is 0 to 3 and R^3 is as defined above.

18. The pharmaceutical composition of claim 17 wherein in the compound B is O.

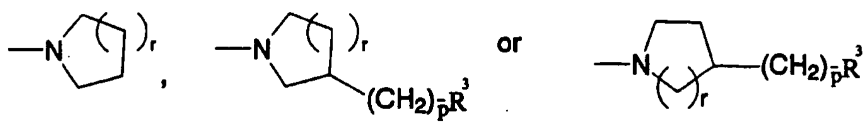
19. The pharmaceutical composition of claim 17 wherein in the compound A is phenyl.

20. The pharmaceutical composition of claim 18 wherein in the compound A is

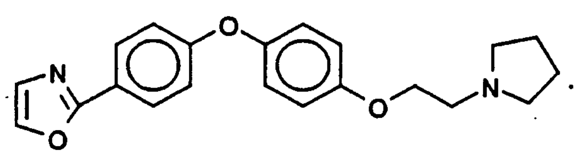


21. The pharmaceutical composition of claim 18 wherein in the compound Y is O and q is 1.

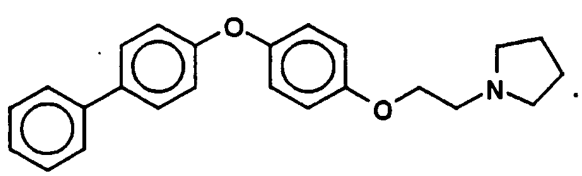
22. The pharmaceutical composition of claim 18 wherein in the compound NR^1R^2 is combined to form



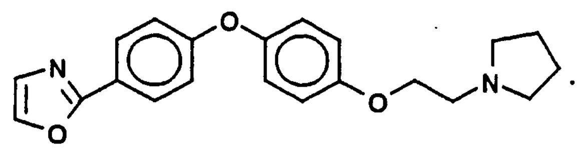
23. The pharmaceutical composition of claim 17 wherein the compound has the structure:



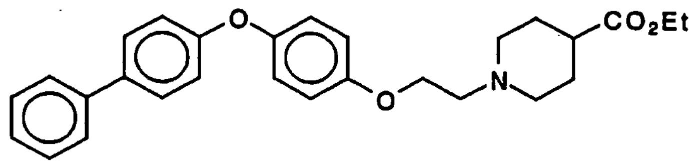
24. The pharmaceutical composition of claim 17 wherein the compound has the structure:



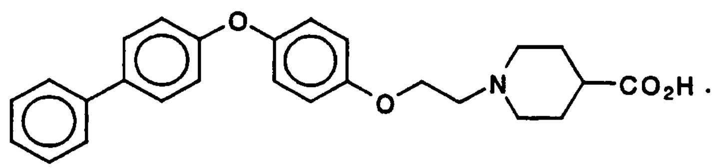
25. The pharmaceutical composition of claim 17 wherein the compound has the structure:



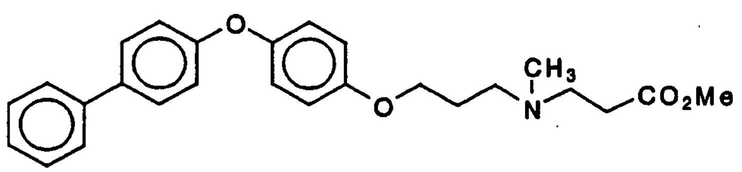
26. The pharmaceutical composition of claim 17 wherein the compound has the structure:



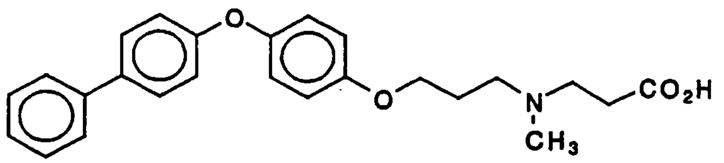
27. The pharmaceutical composition of claim 17 wherein the compound has the structure:



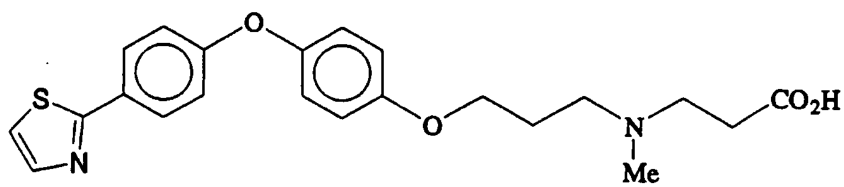
28. The pharmaceutical composition of claim 17 wherein the compound has the structure:



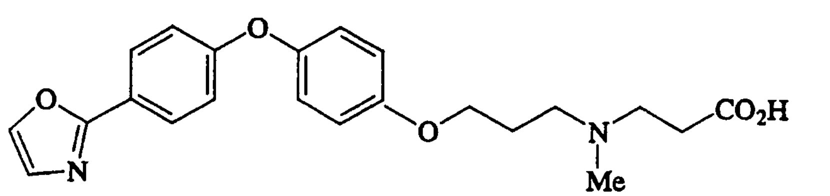
29. The pharmaceutical composition of claim 17 wherein the compound has the structure:



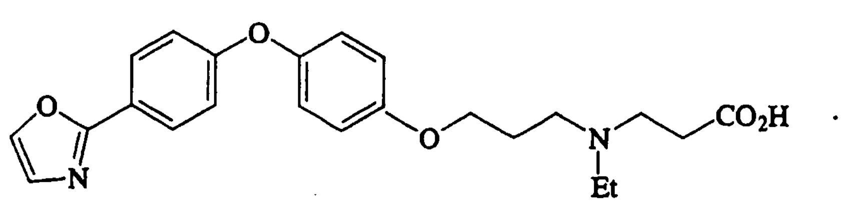
30. The pharmaceutical composition of claim 17 wherein the compound has the structure:



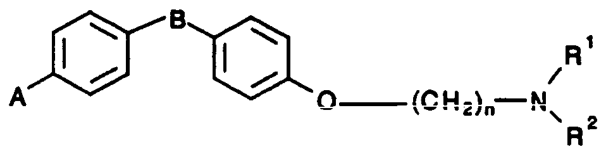
31. The pharmaceutical composition of claim 17 wherein the compound has the structure:



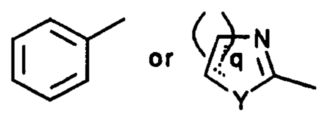
32. The pharmaceutical composition of claim 17 wherein the compound has the structure:



33. A method for treating an LTB_4-mediated inflammatory disease comprising administering to a mammal in need of treatment a therapeutically effective amount of a compound having the structure:



or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable carrier, wherein

A is 

wherein ..... represents a single or double bond

q is 1 or 2, and

Y is -O-, -S- or -CH-

B is -O-, $-CH_2-$ or $-CH_2O-$

n is 2 to 4

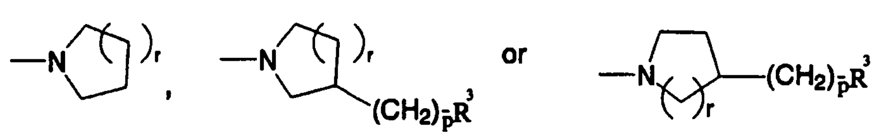
R^1 is H or C_1 to C_4 alkyl

R^2 is $(CH_2)_m$ R^3 wherein n is 1 to 3

R^3 is CO_2R^4

R^4 is H alkyl, amino, alkylamino, dialkylamino

or NR^1R^2 is combined to form

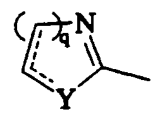


wherein r is 1 or 2, p is 0 to 3 and R^3 is as defined above.

34. The method of claim 33 wherein in the structure of the compound B is O.

35. The method of claim 34 wherein in the structure of the compound A is phenyl.

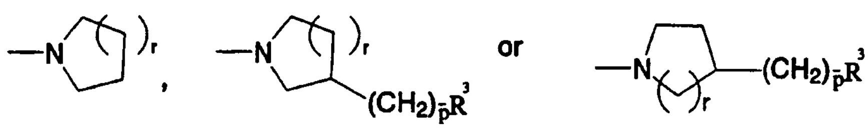
36. The method of claim 34 wherein in the structure of the compound A is



.

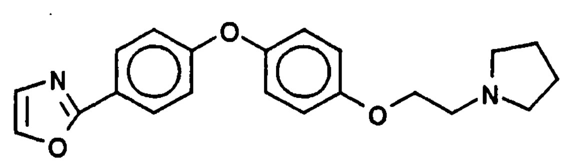
37. The method of claim 34 wherein in the structure of the compound Y is O and q is 1.

38. The method of claim 34 wherein in the structure of the compound NR^1R^2 is combined to form



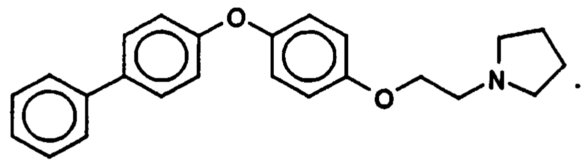
.

39. The method of claim 33 wherein the compound has the structure:

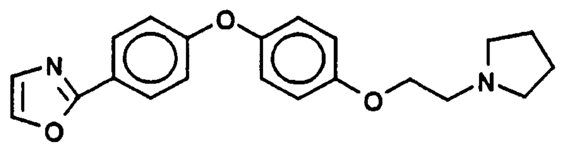


.

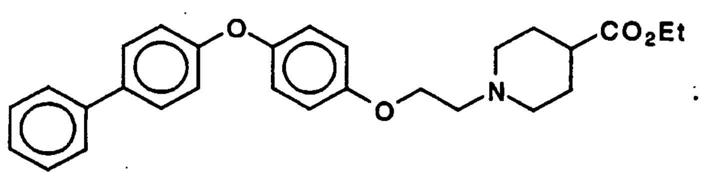
40. The method of claim 33 wherein the compound has the structure:



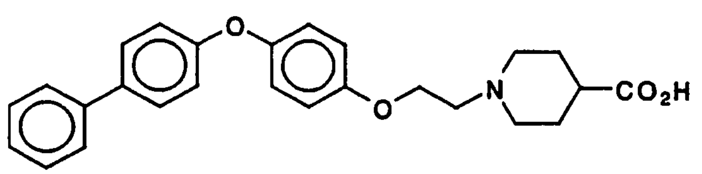
41. The method of claim 33 wherein the compound has the structure:



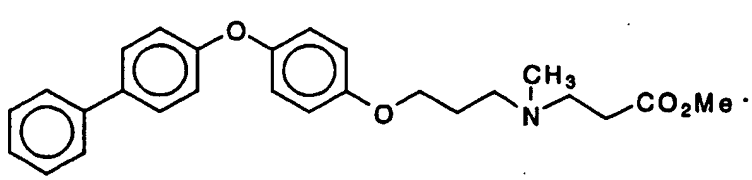
42. The method of claim 33 wherein the compound has the structure:



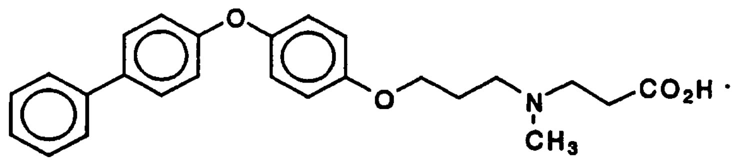
43. The method of claim 33 wherein the compound has the structure:



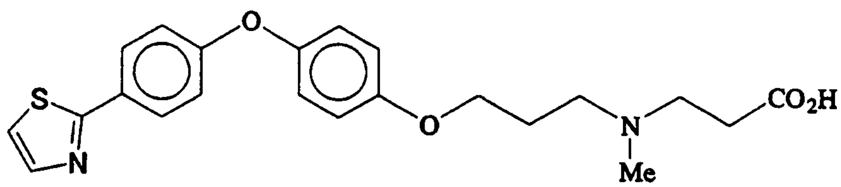
44. The method of claim 33 wherein the compound has the structure:



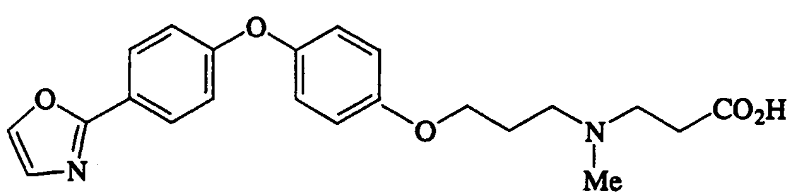
45. The method of claim 33 wherein the compound has the structure:



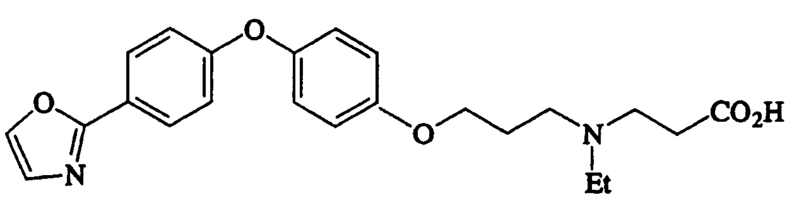
46. The method of claim 33 wherein the compound has the structure:



47. The method of claim 33 wherein the compound has the structure:



48. The method of claim 33 wherein the compound has the structure:



INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 98/03926

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D263/32 A61K31/445 A61K31/42 A61K31/425 A61K31/40
A61K31/195 C07D295/08 C07D211/62 C07D277/24 C07C229/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 41625 A (SEARLE & CO) 27 December 1996 see abstract; claims; tables A,,1,,2 see page 22 ---	1-48
A	WO 96 11192 A (SEARLE & CO ;CHANDRAKUMAR NIZAL SAMUEL (US); CHEN BARBARA BAOSHENG) 18 April 1996 see abstract; claims; examples 267,268,272 -----	1-48

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 June 1998	22.06.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Paisdor, B

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/03926

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 33 - 48
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/03926

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9641625 A	27-12-1996	US 5700816 A	23-12-1997
		AU 6274496 A	09-01-1997
		EP 0843549 A	27-05-1998
WO 9611192 A	18-04-1996	US 5585492 A	17-12-1996
		AU 3686595 A	02-05-1996
		CA 2202371 A	18-04-1996
		EP 0804427 A	05-11-1997
		US 5719306 A	17-02-1998

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